

In the claims:

1-32. (Canceled)

33. (Previously presented) A method of silencing a target gene in an organism by post-transcriptional gene silencing (PTGS), the method comprising the step of introducing into the organism a silencing agent which targets a targeted region of said target gene,

wherein the silencing agent comprises short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which are specific for the targeted region of the target gene.

34. (Previously presented) The method of claim 33 wherein said silencing agent consists of said short RNA molecules.

35. (Previously presented) The method of claim 33 wherein said silencing agent comprises short RNA molecules which are 21 to 25 nucleotides in length.

36. (Previously presented) The method of claim 33 wherein said silencing agent consists of short RNA molecules which are 21 to 25 nucleotides in length.

37. (Previously presented) The method of claim 33 wherein said SRMs are short anti-sense RNA molecules (SARMS) and/or short sense RNA molecules (SSRMS).

38. (Previously presented) The method of claim 33 wherein said SRMs are short anti-sense RNA molecules.

39. (Previously presented) The method of claim 33 wherein said SRMs are short sense RNA molecules.

40. (Previously presented) A method of silencing a target gene in an organism, comprising

(a) providing a DNA construct containing a promoter operably linked to a DNA which upon transcription in a host cell results in a silencing agent specific to a target gene, wherein the silencing agent comprises one or more short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which silencing agent is specific for a targeted region in a target gene,

(b) introducing said construct into said organism such that the target gene in the organism is silenced by the silencing agent transcribed by said promoter.

41. (Previously presented) The method of claim 40 wherein said silencing agent comprises short RNA molecules 21 to 25 nucleotides in length.

42. (Previously presented) The method of claim 40 wherein said silencing agent comprises short RNA molecules (SRMs) wherein said SRMs are short antisense RNA molecules (SARMs) and/or short sense RNA molecules (SSRMs).

43. (Previously presented) The method of claim 42 wherein said SRMs are SARMs.

44. (Previously presented) The method of claim 42 wherein said SRMs are SSRMs.

45. (Previously presented) A host cell containing a DNA construct which comprises a promoter operably linked to DNA which upon transcription in the host cell results in a silencing agent specific to a target gene, and wherein the silencing agent comprises one or more short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which SRMs are specific for a targeted region in a target gene and upon transcription silence the target gene.

46. (Previously presented) A method of selecting a target region in a target gene which is desired to be silenced-comprising:

(I) isolating one or more RNA molecules from a sample, wherein said RNA molecules are short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which are specific for a target region of a target gene by:

(a) producing a nucleic acid extract from said sample;
(b) purifying said extract to obtain purified RNA molecules by effecting at least one purification step selected from the group consisting of (i) filtration; (ii) differential precipitation and (iii) ion exchange chromatography and isolating SRMs which are silencing agents for said target gene;
(II) identifying a target region in the sequence of said target gene which corresponds to a sequence comprised in said SRMs.

47. (Previously presented) The method of claim 46 which further comprises separating the purified RNA molecules according to size by gel electrophoresis using a 15%

polyacrylamide, gel containing 7M urea as a denaturant and TBE (0.5x) as a buffer.

48. (Previously presented) The method of claim 47 which further comprises transferring the RNA molecules comprised on the gel to a hybridization membrane by electrophoresis.

49. (Previously presented) The method of claim 48 which further comprises labeling the RNA molecules comprised on the hybridization membrane using a radioactive probe obtained from a single stranded RNA molecule transcribed in vitro from a plasmid DNA template.

50. (Previously presented) A method of silencing a target gene in an organism comprising:

- (i) performing a method according to claim 45 to select a target region of a target gene to be silenced; and
- (ii) silencing said target gene in an organism by targeting said target region with a silencing agent.

51. (Previously presented) The method of claim 50 wherein step (ii) is effected by introducing into the organism SRMs specific to the targeted region of the target gene which induce silencing of said target gene.

52. (Previously presented) The method of claim 51 wherein said SRMs comprise RNA molecules which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides.

53. (Previously presented) The method of claim 52 wherein said SRMs comprise RNAs which are 21 to 25 nucleotides in length.

54. (Previously presented) A method of silencing a target gene in a first organism comprising:
(i) generating in a second organism short RNA molecules (SRMs) which are a silencing agent for said target gene, wherein said SRMs are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and are specific for a target region in said target gene; and
(ii) introducing said SRMs into said first organism in order to silence said target gene comprised therein.

55. (Previously presented) The method of claim 54 wherein said SRMs are 21 to 25 nucleotides in length.

56. (Previously presented) The method of claim 54 wherein said SRMs are short anti-sense RNA molecules (SARMs) and/or short sense RNA molecules (SSRMs).

57. (Previously presented) The method of claim 54 wherein said SRMs are SARMs.

58. (Previously presented) The method of claim 54 wherein said SRMs are SSRMs.

59. (Previously presented) The method of claim 54 wherein said target gene is endogenous to the first organism but is not endogenous to the second organism.

60. (Previously presented) A method of inhibiting the translation of a gene product in a cell by post-transcriptional gene silencing by introducing into said cell at least one short RNA molecule (SRM) that is 25 nucleotides

in length plus or minus 1, 2, 3, 4 or 5 nucleotides, wherein said SRM has a sequence complementary to an mRNA that encodes said gene product.

61. (Previously presented) The method of Claim 60 wherein said SRM is 20 to 25 nucleotides in length.

62. (Previously presented) The method of Claim 60 wherein said SRM is 21 to 24 nucleotides in length.

63. (Previously presented) The method of Claim 60 wherein said SRM is 21 to 23 nucleotides in length.

64. (Previously presented) The method of Claim 60 wherein said SRM does not affect transcription of mRNA encoding said gene product.

65. (Previously presented) The method of Claim 60 wherein said SRM is the only exogenously introduced RNA that inhibits the translation of said gene product.

66. (Previously presented) The method of Claim 60 wherein said introduced SRM is transcribed from a DNA vector or construct introduced into said cell.

67. (Previously presented) The method of Claim 66 wherein said vector or construct is stably maintained by said cell.

68. (Previously presented) The method of Claim 67 wherein said vector or construct transcribes one or more SRMs.

69. (Previously presented) The method of Claim 60 wherein said cell is from an organism selected from the group consisting of a plant, mammal, insect, avian, reptile, protozoan and nematode.

70. (Previously presented) The method of Claim 69 wherein said cell is comprised in a non-human organism.

71. (Previously presented) The method of Claim 60 wherein said SRM silences a target gene in said cell.

72. (Previously presented) The method of Claim 71 wherein said target gene is endogenous to said cell.

73. (Previously presented) The method of Claim 71 wherein said target gene is selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, a neurological process and signal transduction.

74. (Previously presented) The method of Claim 71 wherein said target gene is selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

75. (Previously presented) The method of Claim 71 wherein said target gene is expressed by a virus, parasite or predator of an organism containing said target gene.

76. (Previously presented) The method of Claim 71 wherein said target gene is involved in parasite resistance.

77. (Previously presented) The method of claim 60 wherein said SRM comprises short sense and antisense molecules complementary to a sequence contained in a gene that encodes said gene product.

78. (Previously presented) The method of claim 77 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

79. (Previously presented) The method of Claim 60 wherein said cell is comprised in an organism.

80. (Previously presented) The method of Claim 79 wherein said organism is selected from the group consisting of a plant, mammal, avian, reptile, insect, protozoan, and a nematode.

81. (Previously presented) The method of Claim 80 wherein said organism is a mammal.

82. (Previously presented) The method of Claim 81 wherein said organism is a rodent.

83. (Previously presented) The method of Claim 80 wherein said organism is a plant.

84. (Previously presented) A method of selectively silencing a target gene in a cell comprising introducing an exogenous nucleic acid into said cell, wherein said exogenous nucleic acid comprises:

(a) a transcribable nucleic acid construct encoding a SRM or a precursor of a SRM, wherein said SRM is

characterized as a short RNA molecule 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides and which has a nucleic acid sequence complementary to a portion of said target gene; and

(b) a transcriptional promoter upstream from said sequence encoding said SRM, wherein said promoter is selectively only active in a specific cell type, or is only active in a specific cell type, or is only active in response to an externally controllable stimulus.

85. (Previously presented) The method of Claim 84 wherein said cell is selected from the group consisting of plant, mammalian, insect, nematode, avian, reptilian, and nematode cell.

86. (Previously presented) The method of Claim 84 wherein the target gene is endogenous to said cell.

87. (Previously presented) The method of claim 84 wherein said target gene is selected from the group consisting of genes involved in cancer, apoptosis, cell-cycle regulation, neurological processes and signal transduction.

88. (Previously presented) The method of Claim 84 wherein said target gene is selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member genes.

89. (Previously presented) The method of Claim 84 wherein said target gene is expressed by a virus, parasite or predator that affects an organism which contains said cell.

90. (Previously presented) The method of Claim 84 wherein said SRM is transcribed by a vector or construct stably integrated into said cell.

91. (Previously presented) The method of claim 84 wherein said at least one SRM comprises sense and antisense RNA molecules which are complementary to a portion of said target gene.

92. (Previously presented) The method of claim 91 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

93. (Previously presented) A method of introducing systemic PTGS of a target gene in an organism which comprises introducing into said organism an SRM or a transcribable nucleic acid construct encoding a SRM wherein said SRM is characterized as a short RNA molecule 25 nucleotides in length, plus or minus 1, 2, 3, 4 or 5 nucleotides and which has a nucleic acid sequence complementary to a portion of said target gene.

94. (Previously presented) The method of Claim 93 wherein said target gene is endogenous to said cell.

95. (Previously presented) The method of Claim 93 wherein said target gene is selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, a neurological process and signal transduction.

96. (Previously presented) The method of Claim 93 wherein said gene is selected from group the consisting of an

oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

97. (Previously presented) The method of Claim 93 wherein said target gene is comprised in a virus, parasite or predator affects an organism containing said gene.

98. (Previously presented) The method of Claim 93 wherein said cell is selected from the group consisting of a plant, mammalian, insect, nematode, avian, reptilian, and protozoan cell.

99. (Previously presented) The method of Claim 93 wherein said SRM is transcribed by a vector or DNA construct introduced into said cell.

100. (Previously presented) The method of claim 93 wherein said SRMs comprise short sense and antisense RNA molecules which are complementary to a portion of said target gene.

101. (Previously presented) The method of claim 100 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

102. (Previously presented) A method of inducing post transcriptional gene silencing in a cell which comprises introducing into said cell a selected nucleic acid sequence wherein said nucleic acid is selected for introduction into said cell based on a finding that said nucleic acid when introduced into an equivalent cell induces the production in said cell of short RNA molecules, said short RNA molecules characterized in that they are 25 nucleotides in length, plus

or minus 1, 2, 3, 4 or 5 nucleotides, wherein said nucleic is sufficiently complementary in sequence specificity to a mRNA otherwise present in said cell to interfere with the stability and translation of said mRNA.

103. (Previously presented) The method of Claim 102 wherein said RNA molecules are transcribed by a vector or construct stably integrated into said cell.

104. (Previously presented) The method of Claim 102 wherein said cell is selected from the group consisting of a plant, a mammal, an avian organism, a reptile, an insect, a nematode, and a protozoan cell.

105. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a target gene endogenous to said cell.

106. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a gene selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, neurological processes and signal transduction.

107. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a gene selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

108. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a gene of a parasite or predator that affects an organism containing said cell.

109. (Previously presented) The method of claim 102 wherein said SRMs comprise short sense and antisense RNA molecules which are complementary to a gene which transcribes said mRNA.

110. (Previously presented) The method of claim 109 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.